

**848-Pos Board B603****Novel Kinesin Regulators of Gamma-TuRC**

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Mitotic spindle assembly is a critical control point in eukaryotic chromosome segregation. In fission yeast conserved mitotic kinesin-like proteins (Klps) Kinesin-14 and Kinesin-5 are emerging as novel regulators of the  $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC) MTOC. While these Klps typically participate in microtubule roles for spindle assembly, alternatively in fission yeast they localize to  $\gamma$ -TuRC and when co-deleted a bipolar spindle forms. Our analysis by genetics, biochemistry, cell biology and nanotechnology provides new knowledge on mitotic mechanisms in *S. pombe*. Previous work from our lab and others uncovered a novel functional relationship between Kinesin-14 Pkl1 and all proteins of the  $\gamma$ -tubulin small complex ( $\gamma$ -TuSC). We demonstrated that Pkl1 binds  $\gamma$ -TuRC through Motor and Tail interactions to directly regulate its function. A Pkl1 Tail peptide is sufficient in vitro and disrupts  $\gamma$ -TuRC structure by removal of  $\gamma$ -tubulin subunits (Cell Cycle 2013), an action that is reversible. New data from our lab indicates that the essential Kinesin-5 Cut7 is actually dispensable in the absence of Pkl1 with which it shares an important antagonistic MTOC regulatory relationship. Cut-7 associates with FPLC purified  $\gamma$ -TuRC and binds to  $\gamma$ -tubulin similarly as Pkl1. We demonstrate in vivo that Pkl1 blocks microtubule nucleation from  $\gamma$ -TuRC and additionally influences spindle microtubule numbers and microtubule overlap. Kinetochore-based microtubule motors Klp5 and Klp6 may facilitate spindle organization, since triple mutants of Pkl1, Cut7 and either Kinesin-6 Klp9 or Kinesin-14 Klp2 are viable. Previously we showed in vivo that human Kinesin-14 HSET, but not *Drosophila* Ncd, functionally replaces Pkl1 and more recently that human  $\gamma$ -TuSC proteins GCP2 and GCP3 replace fission yeast Alp4 and Alp6 (JCS 2013). Our research highlights conserved in vivo functions of  $\gamma$ -TuSC and provides a new model of spindle assembly in *S. pombe*.

**849-Pos Board B604****Measurement of the Force that Centers the Mitotic Spindle in the Early *C. elegans* Embryo using Magnetic Tweezers**

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Because the plane of cell division bisects the mitotic spindle, the positioning of the spindle plays a key role in specifying the size of the daughter cells and in segregating cytoplasmic constituents. Little is known, however, about the mechanical processes underlying spindle positioning. To study this mechanism, we built an apparatus whereby calibrated magnetic forces are applied to the spindle via super-paramagnetic beads inserted into the cytoplasm of one- and two-cell *C. elegans* embryos.

At metaphase in the one-cell embryo, a 20 pN force displaced the mitotic spindle pole of one-cell embryos in metaphase approximately 1  $\mu$ m away from the anterior-posterior axis over 10-20 seconds. By tracking the bead displacement, we found that the spindle behaved roughly as a damped spring with a spring constant of  $22 \pm 13$  pN/ $\mu$ m and a drag coefficient of  $138 \pm 71$  pN·s/ $\mu$ m (mean  $\pm$  SD, 26 traces from 19 cells). The stiffness was five-fold higher during anaphase, indicating that the centering forces increase during the cell cycle. The stiffness was two-fold higher in the two-cell embryo, consistent with a centering mechanism that scales inversely with cell size. Finally, gpr-1/2 RNAi knockdown had no significant effect on the viscoelastic properties of either metaphase or anaphase spindles.

Taken together, our results constrain molecular models of centering. The gpr-1/2 results rule out a role for cortical forces pulling on the spindle via astral microtubules. On the other hand, the results are consistent with centering being mediated by astral microtubules either pushing against the cortex or being pulled by cytoplasmic factors.

**850-Pos Board B605****Single-Molecule Investigation of Intraflagellar Transport Dynamics at the Flagellar Tip**

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In the past decade, cilia/flagella have come to be known as essential sensory organelles for cells. They perform their sensory duties via signaling pathways comprised of transmembrane signaling proteins (TSP) and intraflagellar

transport (IFT) machineries that are responsible for translocating TSPs within the flagellum. While IFT average speed, IFT train frequency, and IFT train size have already been studied, little is known about IFT dynamics at the flagellar tip region where anterograde IFT trains deposit their cargo and rearrange for retrograde transport to the cell body. To address this issue, we use single-molecule fluorescence imaging methods to study the motion of GFP-tagged BBS4, an IFT-associated protein that is part of the BBSome complex, at the flagellar tip in *Chlamydomonas reinhardtii*. These investigations have yielded the following results: (i) In the tip region, BBSomes remain attached to their cargo TSPs and diffuse along the flagellar membrane with a diffusion coefficient of 1130 nm<sup>2</sup>/s. This result contrasts with the current understanding that the BBSome either remains bound to IFT train on the microtubule or diffuses in the flagellar lumen. (ii) On average, BBSomes remain at the flagellar tip for 2.6 seconds before undergoing retrograde IFT. (iii) Our BBS4-GFP image-size investigation has helped further uncover the nature of the BBSome's oligomerization on IFT trains both during active transport along the flagellum and during the turn-around period at the flagellar tip.

**851-Pos Board B606****The Role of Cdc42 and Gic1 in the Regulation of Septin Filament Formation and Dissociation**

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Septins are guanine nucleotide-binding proteins that polymerize into filamentous and higher-order structures. Cdc42 and its effector Gic1 are involved in septin recruitment, ring formation and dissociation. The regulatory mechanisms behind these processes are not well understood. Here, we have used electron microscopy and cryo electron tomography to elucidate the structural basis of the Gic1-septin and Gic1-Cdc42-septin interaction. We show that Gic1 acts as a scaffolding protein for septin filaments forming long and flexible filament cables. Cdc42 in its GTP-form binds to Gic1, which ultimately leads to the dissociation of Gic1 from the filament cables. Surprisingly, Cdc42-GDP is not inactive, but in the absence of Gic1 directly interacts with septin filaments resulting in their disassembly. We suggest that this unanticipated dual function of Cdc42 is crucial for the cell cycle. Based on our results we propose a novel regulatory mechanism for septin filament formation and dissociation.

**852-Pos Board B607****Focal Adhesions are Composed of Filamentous Subunits Whose Length and Dynamics Depend on the Cell Spreading Area**

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The cells ability to adhere to the extracellular matrix (ECM) is a fundamental feature of many higher eukaryotic cells and is required for cell migration, proliferation, and differentiation. At the scale of single cells, the primary patterns of adhesion to the ECM are called focal adhesions (FAs). They are thought to serve as mechano-sensor units. Recent progress in super resolution techniques opened the door to study ultrastructure of focal adhesions. However the dynamics of their spatial structure has not been studied yet. Here, we combine structured illumination microscopy (SIM) with total internal reflection fluorescence microscopy (TIRF) to study focal adhesions at high spatial resolution in live cells. We studied REF52 fibroblasts spread on fibronectin disks to avoid the variability of focal adhesions properties due to variable cell morphology and migratory status. We observed the formation of many focal adhesions localized at the lamellipodium-lamellum interface. We further show that (i) focal adhesions are formed by filaments subunits which grow retrograde and then shrink in  $\sim 20$  mins, (ii) that their formation depends on the spreading area of the cell and (iii) that each filament connects to a single actin cable, therefore linking the internal structure of focal adhesions to the maturation process through actin pulling on the focal complex. Thus our study reveals the ultrastructure and dynamics of focal adhesion and is a first step towards a better understanding of the formation of FAs with respect to the cell mechanical state.

Keywords: focal adhesions, filamentous unit, SIM, retrograde flow, spreading area, paxillin.